

TOTAL PHENOLIC AND TOTAL FLAVONOIDS CONTENT OF PITAYA PEELS BY
WATER EXTRACTION

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I declare that this thesis is entitled “*Total Phenolic and Total Flavonoids Content of Pitaya Peels by Water Extraction*” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :

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Date : 8 APRIL 2009

Special dedication to my family members, my supervisor, staffs of FKKSA laboratory
and all my beloved friends.

For all your love and support. Thank you very much.

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ABSTRACT

Residues from the processing of fruits and vegetables which are traditionally considered an environmental problem is now widely recognized for obtaining high-phenolic products. This paper was designed to study the total phenolic and total flavonoids contents in the pitaya peels. The total phenolic content was determined by using the Folin-Ciocalteu assay while the total flavonoids was measured using aluminum chloride colorimetric assay by UV-visible spectrometer. The result showed that the highest total phenolic content in pitaya peels was extracted at the optimum dose of aluminum sulfate concentration of 25 mg/L (3.32 mg gallic acid equivalents (GAE)/ 25 g at 80°C, 3.21 mg GAE/25g at 60°C and 1.73 mg GAE/25g at 40°C). The greatest total flavonoids content in pitaya peels was extracted (2.24 mg catechin equivalents (CE)/25 g at 60°C, 1.79 mg CE/25g at 80°C and 1.60 mg CE/25g at 40°C) at the concentration of aluminum sulfate of 30 mg/L. The results showed that the value of the total phenolic content decreased when the concentration of aluminum sulfate of 25 mg/L due to the fact that the high concentration of aluminum sulfate (more than 25 mg/L) could have reacted with the phenolic compound of the pitaya peels to form another compound. The total flavonoid was found to be extracted with the highest value at temperature of 60°C and the lowest at 80°C. This shows that the flavonoids compound was somehow destroyed at temperature of 80°C.

ABSTRAK

Sisa pemrosesaan buah-buahan dan sayur-sayuran yang selalunya menjadi punca masalah alam sekitar kini semakin mendapat perhatian ramai kerana sisa tersebut mengandungi jumlah kandungan fenolik yang memberangsangkan. Kajian ini bertujuan untuk mengenal pasti jumlah kandungan fenolik dan jumlah kandungan *flavonoids* yang terkandung dalam kulit buah naga. Jumlah kandungan fenolik ditentukan melalui kaedah *Folin-Ciocalteu*, manakala jumlah kandungan *flavonoids* pula ditentukan melalui kaedah kalorimetri aluminium klorida dengan bantuan alat UV-Vis spectrometer. Keputusan kajian menunjukkan bahawa jumlah kandungan fenolik yang tertinggi dalam kulit buah naga ialah 25 mg/L sebanyak (3.32 asid gallic bersamaan (GAE/ 25 g pada 80°C, 3.21 mg GAE/ 25g pada 60°C dan 1.73 mg GAE/ 25g pada 40°C) iaitu pada aluminium sulfate yang optimum. Bagi jumlah kandungan *flavonoids* yang dapat dalam kulit buah naga, adalah sebanyak (2.24 mg *catechin* bersamaan CE/25g pada 60°C, 1.79 mg CE/25g pada 80°C dan 1.60 mg CE/ 25g pada 40°C) pada kepekatan aluminium sulfate 30 mg/L. Keputusan juga menunjukkan kecerunan graf pada setiap suhu bagi jumlah kandungan fenolik adalah menurun selepas 25 mg/L. Ini berkemungkinan adalah asid yang berkepekatan tinggi (selepas kepekatan 25 mg/L) bertindakbalas dengan jumlah kandungan fenolik komponen menjadikan ianya berubah kepada komponen yang berlainan. Bagi jumlah kandungan *flavonoids*, penghasilannya semakin bertambah apabila kepekatan aluminium sulfate dicampurkan. Walaubagaimanapun, jumlah kandungan *flavonoids* hanya menunjukkan hasil yang banyak pada suhu 60°C dan bukan pada suhu 80°C. Ini menunjukkan jumlah kandungan *flavonoids* telah diubahkan dari segi struktur pada suhu 80°C dan mengakibatkan komponen itu menjadi bahan yang lain.

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LIST OF ABBREVIATIONS/SYMBOLS

%	-	Percent
AlCl ₃	-	Aluminum chloride
CE	-	Catechin equivalent
F-C	-	Folin-Ciocalteu
G	-	Gram
g/L	-	Gram per liter
GAE	-	Gallic acid equivalent
kg	-	kilogram
L	-	Liter
mg/L	-	Milligram per liter
Na ₂ CO ₃	-	Sodium carbonate
NaNO ₂	-	Sodium nitrate
NaOH	-	Sodium hydroxide
nm	-	Nanometer
°C	-	Degree celsius
TF	-	Total flavonoids
TPC	-	Total phenolic content
UV-Vis	-	Ultra Violet-Visible
Vs	-	Versus
μL	-	Micro liter

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Phenolic compounds are secondary metabolites of plants. They are naturally present in fruits and vegetables. These compounds are a part of the everyday diet and also used as medicines or supplements. Research has shown that fruits and vegetables contain other antioxidant nutrients, in addition to vitamins C and E, and carotenoids, which significantly contribute to their total antioxidant capacity. The major part of those antioxidant nutrients is polyphenolic compounds, which are components of fruits and vegetables having strong antioxidant capacity. Flavonoids have a wide range of biological activities, such as cell-proliferation-inhibiting, apoptosis-inducing, enzyme-inhibiting, antibacterial, and antioxidant effects. Moreover, some findings indicate that flavonoids possess various clinical properties, such as antiatherosclerotic, anti-inflammatory, antitumour, antithrombogenic, antiosteoporotic, and antiviral effects (Su *et al.*, 2008).

Since ancient times, the ability of natural dyes to color had been known. In the earliest written record, the use of natural dyes was found in China dated 2600 BC.

Another proof to the use of natural dyes in ancient time is by the 4th century AD, dyes such as woad, Brazilwood and etcaeteras where Brazil was named for the wood found there. Natural dyes can be obtained from plants, animals and minerals. For example, the turkey red was extracted from madder plant was so popular and used for dyeing until the mid 1800s.

Pitaya (*Hylocereus undatus*(Haw)) or dragon fruit which have been planted in Viet Nam for almost 100 years (Mizrahi *et al.*, 1997). Nowadays, coloring foodstuffs are the most popular way to achieve desired hues because of their application does not require certification (Stich *et al.*, 1999; Stintzing *et al.*, 2004). In addition, natural dyes from the plants or fruits are believed to be safe as they are non-toxic, non-carcinogenic and biodegradable nature. So, natural dyes are widely produced nowadays from natural resources like from the plants or fruits which its colors can be extracted through various types of method. It is then safe to be used after detoxification process.

1.2 Problem Statement

Polyphenol which it is widely used for the antioxidants are normally used in fats, oils, soap and cosmetics to prevent oxidative rancidity. Other than the natural antioxidants, artificial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are extensively used in food and some commercial products. Recently, there has been a study finding out that those artificial antioxidants may cause loss of nourishment and even produce toxic substances to harm people's health. Hence, phenolic antioxidants are strictly controlled for its use with the definition of the amounts of these additives to be allowed added in foods. Thus, it is

crucial to find out more natural phenolic to supply to the needs of phenolic from the natural sources. (Yong *et al.*, 1999)

The present trend throughout the world is shifting towards the use of eco-friendly and biodegradable commodities, ranging from food industry to textile industry. Hence, the demand for natural dyes is increasing day by day (Bhuyan *et al.*, 2004).

Recent studies by the European Union found out that the colouring, Red 2G which is being used in all types of food especially in enhancing the colour of burgers and sausages that could endanger the health of humans like destroying the genetic material in cells and causing cancer has been banned with immediate effect by The Health Ministry of Malaysia while EU had banned this type of colouring on July 27, 2007 (The Star, 2007). The Sudan Red is widely used for colouring solvents, oils, waxes and floor polishes which is illegally used to enhance and maintain the colour of the food products has been prohibited for use in food products by Regulations Relating to Food Colorants (R.1008) of the Foodstuffs, Cosmetics and Disinfectant Act 54 of 1972 in South Africa. Research indicates that the Sudan Red brings carcinogenic effect to human beings, and genotoxic effect which is capable of causing damage to DNA.

While in textile industry, it can mostly produce and use approximately 1.3 million tones of dyes, pigments and dye precursors, valued at around \$ 23 billion, almost all of which is manufactured synthetically. However, synthetic dyes have some limitations. First, the production process requires hazardous chemicals, creating worker safety concerns. Second, they may generate hazardous wastes and third, these dyes are not environment friendly (Sengupta *et al.*, 2003). So in the textiles industry nowadays, there is a revival of natural dyes in textiles is growing interest because of the green chemistry, improved eco-balances and thereby leading to the niche products for special markets (Bechtold *et al.*, 2006).

Consumers nowadays are more aware of the poisonous of consuming artificial dyes where it can lead to fatal and demanding for natural product or natural sources as natural dyes lack of toxicity during production and having full biodegradation and reduction of the environmental impact.

1.3 Significance of Study

Phenolic compounds are secondary metabolites in plants. Over the past 10 years, there has been increasing interest in phenolic compounds and their role in human health and nutrition. Some phenolic compounds present in natural products have higher antioxidant activities than those of synthetic antioxidants. These polyphenolic antioxidants can also be used to preserve foods because of their protective effects against microorganisms.

Residues from the processing of fruits and vegetables, traditionally considered as an environmental problem, are being increasingly recognized as sources for obtaining high-phenolic products. The polyphenolics from waste materials deriving from agro-industry production may be used as functional food ingredients and as natural antioxidants to replace their synthetic equivalents that have experienced growing rejection. (Zhou *et al.*, 2008)

Natural dyes can offer not only a rich and varied source of dyestuff, but also the possibility of an income through sustainable harvest of the dye plants. Another aspect to be considered by an imaginary supplier of natural dyes has been identified during an extensive study of possible future use of natural dyes, namely that the plant material

which contains the natural dye needs the same level of standardization as modern synthetic dyes already have achieved at present (Ganglberger *et al.*, 2003; Rappl, 2005).

Furthermore there is abundance of waste contributed by certain industries like in Food industry which produces cans of Pitaya leaving the Pitaya peels which can be fully utilized to be extracted for its natural red dyes by using aqueous extraction method. Hence producing of natural dyes are much more to be encouraged to fulfill the demand of the market and reduce the waste disposal.

Production of red natural dye from Pitaya waste is natural and its red colourants are now growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes (Duhard *et al.*, 1997). As we know the plants have generally produced less amount of colouring component on extraction which do not sufficient to provide for the needs of market. Also, there is a restriction in using the natural dyes in comparison with synthetic dyes because of the cost of the yield of natural dyes (Ali *et al.*, 2008). With the appearance of synthetic dyes the use of natural dyes for textile dyeing almost disappeared. The wide range of colours available with good fastness properties at moderate costs was the main reason for the replacement of natural dyes by their synthetic counterparts.

Hence, it is imperative to optimize more colouring component with keeping the environment safe through alkaline extraction from Pitaya waste excluding any hazardous application of organic solvents.

The present work is, therefore, undertaken to extract more colouring components with keeping the environment friendly extraction procedure excluding the extensive application of organic solvents.

1.4 Objectives

The current research is done to achieve the following objectives:

- i) To determine the effect of temperature and the concentration of the aluminum sulfate to the extraction of pitaya peels.
- ii) To determine the amount of total phenolic and total flavonoids content exist in the pitaya peels.

1.5 Scope of Study

The scopes of the study are:

- i) Only Red Pitaya peels are used in the experiment.
- ii) Aqueous extraction is involved in the experiment.
- iii) Pitaya waste is heated in 250ml for 4 hours in the water with temperature different at 40°C, 60°C and 80°C.
- iv) Pitaya waste is heated in 125ml at second heating stage for 2 hours with temperature different at 40°C.
- v) The aqueous extraction involves using Aluminum Sulfate at different concentration (mg/L) for 10, 15, 20, 25 and 30 mg/L.

- vi) 80% methanol is used in methanolic extraction.
- vii) Total Phenolic Content (TPC) is determined by using Folin-Ciocalteu method.
- viii) Total flavonoid content (TF) is determined by using the Catechin method.
- xi) Each sample of TPC (Total Phenolic Content) and TF (Total Flavonoids) is determined by UV-visible spectrometer.

CHAPTER 2

LITERATURE REVIEW

2.1 Pitaya

Pitaya (*Hylocerus undatus* (Haw.)) or Dragon Fruit is native to the Central America which has been documented in Aztec literature since the 13th century. It was then commercially grown in Colombia, Nicaragua and Vietnam (LJ Health Promotions, 2003). This fruit was then brought in by the French. Pitaya have been planted in Viet Nam for more than 100 years, following their introduction by the French (Mizrahi *et al.*, 1997). In the local, dragon fruit is named as Thanh Long or in English, it is 'Green Dragon'. The green color is its immature color before ripening. While the 'dragon' is used to name the fruit as its appearance has 'dragon-like' scales or bracts on the surface. Dragon fruit is considered to be the member of *Cactaceae*. The trailing cladodes, stems modified to act as leaves, bear spectacular ovoid fruit year-round which are a bright red colour when it is ripened or becomes mature. The fruit contains white, crimson or dark red color, or some with pale-yellow flesh depending on the cultivar and the flesh is interspersed with small black seeds (Hoa *et al.*, 2006).

2.1.1 Types of Pitaya

2.1.1.1 Yellow Pitaya

Yellow Pitaya (*Selenicereus megalanthus*) is a vine cactus that needs a trellis system for support. In Israel net-houses are required to avoid photo-inhibition and bleaching of its stems. Colombia was the first country to sell *S. megalanthus* in the world market under the name of “yellow pitaya”. This plant can tolerate high temperatures more than the other vine cacti, yields spiny fruits where the spines abscise easily upon ripening (Figure 2.1). The fruits are smaller than the other vine cacti fruits but the taste is superior, hence, the higher prices obtained in the markets in comparison with the others. Most of the plantations in Colombia have been uprooted due to the heavy infestation with fungi.



Figure 2.1 Yellow Pitaya (Wikimedia, 2008)

2.1.1.2 The Red Pitaya

The red Pitaya (*Hylocereus undatus*) is known in Latin America but the Asia name is dragon-fruit (Figure 2.2). There are red flesh clones and some are produced in Nicaragua and are considered *Hylocereus costaricensis*. Pigments differ among the clones and species. For example the *Hylocereus* sp. Clone 10487 has red color while the *H. polyrhizus* clones show glowing purple a unique color which has been chemically identified as hylocerenin and iso-hylocerenin (Mizrahi *et al.*, 2002). Besides, there is the red Pitaya with the peels are in red colored shown in the Figure 2.3 .



Figure 2.2 Red Pitaya with White Flesh (Mizrahi *et al.*, 2002)



Figure 2.3 Red Pitaya with Red Flesh (Mizrahi *et al.*, 2002)

2.1.2 Plant Stem

Pitayas are the fast growing, perennial, terrestrial, epiphytic, and vine-like cacti. The appearance is triangular which are 3-sided, sometimes 4 or 5-sided. It is green, fleshy and having many branched stems. For each of the stem segment, it has 2 flat, wavy wings, with corneous margins and may have 1-3 small spines or be spineless. The stem forms root which is adhered to the surface upon which they grow and the stem may reach about 6.1m long.

2.1.3 Inflorescence

The flowers of the pitaya are hermaphroditic which means a plant having stamens and pistils in the same flower (Oxford Dictionary, 2005). However, some species and cultivars are self incompatible. The showy, edible, white flowers are very large, fragrant, bell shaped and may be 36 cm long and 23 cm wide. The stamens and lobed stigmas are cream colored.

2.1.4 Fruit

The pitaya fruit is fleshy berry, oblong and the thickness is about 11cm with red or yellow peels with scales. The pulp maybe white, red or magenta depending on the species. They are seeds in the pulp which are very small, numerous and black.

2.1.5 Propagation

Pitaya can be propagated from seed, however, the fruit and stem characteristics are variable, and the time from planting to fruit production may be up to around 7 years. Asexual propagation is preferred, and currently, the stem cuttings method is widely applied by most people. The entire stem segments of 12-38 cm are usually used. To make the stem base, the stem has to be cut slanted then being treated with fungicide and dry and heal for 7-8 days in dry, shady location before being planted. Another method which is applying root hormone to the cuttings after curing but before it is done before planting. This cutting method grows very fast and many produce fruit in about 6 to 9 months after planting. Longer cuttings usually reach the trellis supports faster than shorter ones. Another method which is not commonly practiced is the Pitayas may be grafted. Grafting has potential for selection of rootstocks adaptable to various soil types and problems. Cutting takes about 4-6 months to develop a good root system in pots and be ready for planting.

2.2 Polyphenols

Polyphenols are antioxidants in plants that is believed to have a substantial amount of health benefits. Among the most well known of the polyphenols are the flavonoids which are grouping of several thousand individual compounds. These compounds are found together in many different foods, all contributing in a unique way to an individual's overall health. They are most commonly introduced to the body through the consumption of fruits and vegetables.

In addition of flavonoids, other classes of polyphenols include tannins (both condensed tannins and hydrolysable tannins), and lignins. Each of these polyphenols can be found from many different sources. For example, tannins are prevalent in wines, tea and fruits. Nearly all the plants will carry at least some of these polyphenols.

Polyphenols work by eliminating free radicals in the body, which are known to cause a number of health problems. It is thought polyphenols help prevent premature aging and help prevent cancer, among other things. Some, such as tannins, may even have antibiotic benefits as well.

The effect of tannins can be seen in a number of different applications. It is the substance responsible for the browning, or tanning, of leather. In fact, that is how the group of chemicals received their name. In fact, that is how the group of chemicals received their name. Tannic acid can be seen in some lakes and rivers, especially those in the tropical and subtropical areas populated with cypress trees. However, it should be noted that some tannic acid may be harmful to the body.

2.3 Flavonoids

Flavonoids, which is also referred as bioflavonoids, are polyphenol antioxidants found naturally in plants. They are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants. Flavonoids are plant nutrients that when being consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to human body.

Flavonoids are widely disbursed throughout plants. It gives the flowers and fruits of many plants their vibrant colors. They also play a role in protecting the plants from microbe and insect attacks. More importantly, the consumption of foods containing flavonoids has been linked to numerous health benefits. Though research shows flavonoids alone provide minimal antioxidant benefit due to slow absorption by the body, there is indication that they biologically trigger the production of natural enzymes that fight disease.

Recent research indicates that flavonoids can be nutritionally helpful by triggering enzymes that reduce the risk of certain cancers, heart disease, and age-related degenerative diseases. Some research also indicates flavonoids may help prevent tooth decay and reduce the occurrence of common ailments such as the flu. These potential health benefits, many of which have been proven, have become of particular interest to consumers and food manufacturers. Food that contain high amount of flavonoids include blueberries, red beans, cranberries and blackberries. Many of other foods, including red and yellow fruits and vegetables and some nuts, also contain flavonoids. Red wine and certain tea also are rich in flavonoids as well.

2.4 Natural Dye

No chemical dye has the luster, that under-glow of rich colour, the delicious aromatic smell, that soft light and shadow that gives so much pleasure to the eye. These colours are alive (Thurston, 1972). Dyes can be derived from nature through herbs and plants, flowers, seeds, barks and roots. Natural dyes give subtle, rich, warm colors that unique. They have a mystery and life that fascinates and satisfies. Natural dyes can be categorized as either substantive adjective.

2.4.1 Art of Making Natural Dyes History

The art of making natural dyes is one of the oldest known to man and date back to the dawn of Civilization. In India, it was widely used for the colouring of fabrics and other materials. Though the very earliest dyes were discovered by accident using berries and fruits. With the experimentation and gradual development the natural dyes have resulted into a highly refined art.

India's expertise in natural dyes date back to ancient times. Using mordant to hold fast the dyes or resists to selectivity prevent them from touching the cloth were printed bales of whisper soft textiles. From 19th centuries, block printed resist dyes textiles from Gujarat and Deecan adorned Europeans and their homes. The discovery of synthetic dyes in the west in 19th century dealt a massive blow to Indian Textile Industry. Some of the chemical dyes earlier found associated with hazards effecting human life creating skin diseases and lungs problems. The environmentalist, therefore, started searching the substitute of synthetic items which has led the use of more natural dyes. In recent days the inherent advantages of natural dyes has resulted in the revival and use of natural dyes.

2.4.2 Types of Natural Dyes

2.4.2.1 Lac Dye

It is extracted from lacifer lacca insect. It is used for dyeing of wool, silk and cotton fibers. It gives reddish with tin mordant and purplish with copper mordant.

2.4.2.2 Annatto

It is prepared from the seed of annatto. It is used in the dyeing of silk and wool. It gives orange and peach colour. Its botanical name is bixin.

2.4.2.3 Harda

It is prepared from fruits of Harda and it yields yellow and gray colours with aluminum and ferrous mordant respectively. It can be used in coloration of wool and silk.

2.4.2.4 Himalayan Rhubard

It is manufactured from Himalayan herb. The roots of this plants is used fro the manufacture of dye stuff. It gives yellow and oranges. It can be used directly and with Alum Mordant on wool or silk.

2.4.2.5 Indigo Blue

It is a fermented dyes of leaves of indigo ferra tinctoria. It gives blue colour. It can dye cotton, wool and silk.

2.4.2.6 Kamala Dye

It is prepared from the deposits on flowers of Kamala tree. It gives yellow colour on wool and silk. It can be used directly of with mordant as well.

2.4.2.7 Manju Phal

It is manufactured from the nut galls of Manju Phal tree. It is used for dyeing of silk and wool, both directly or with mordant. It gives cream and grey colours with alum and iron mordant.

2.4.2.8 Gum Arabic

It is manufactured from the bark of Indian Gum Arabic tree. It is used for dyeing of cotton with mordant. It yields brown shade having very good fastness.

2.4.2.9 Trigonella Foenum Graecum

It is prepared from the fenugreek seeds. It is used in the dyeing of cotton fabrics. It gives yellow shade with metallic mordents like copper sulphate and ferrous sulphate.

2.4.2.10 Gold Dock

It is prepared from *Rumex maritimus* seeds. It yields brown colour on cotton with alum, copper sulphate and ferrous sulphate mordants.

2.4.3 Uses of Dye

2.4.3.1 Textile

There is a big potential for the use of natural dyes in textile industry. These dyes can be used for coloration of textile material at different stages such as on the yarn, on the fabric and even can be applied on the apparels.

2.4.3.2 Cosmetics

The natural dyes from *Dolichos* and rind of pomegranate are used in coloration of lipsticks and other cosmetics. They are manufactured by water extraction method. The coloring matter can be extracted with Super Critical Fluid Extraction Method.

2.4.3.3 Edible Dyes

Most common dye, which can be used for coloration of the edible items, are annatto seeds. The water soluble extract can be used for coloration of butter and oil soluble extract can be used for colouration of ghee and ice cream. They are simple extracts of annatto seeds, which give 55 to 60% yields when their extract is prepared. Lac dye is also derivative of lac and is similar to cochineal and has been in use for colouring food besides fabrics since ancient times.

2.4.3.4 Food Items

This is the sector where natural dyes can be consumed in appreciable amount. This sector consumes 10 to 20 ton of natural dyes in a year. Moreover, the consumption of natural dyes in beverage sector can be up to 20 to 40 ton per year.

2.4.3.5 Leather

Leather industry is already using natural tannin for tanning of leather sole. However, this use is confined to cottage and small-scale leather units. The large manufacturers are using Chrome Tanning. At present, none of the leather units are using natural dyes for coloration of their product. If sufficient and offensive efforts are done, the natural dyes can capture in leather sector in a big way.

2.5 Extraction of Natural Dyes

The natural dyes extraction is broadly divided into extraction method and extraction technology.

2.5.1 Method of Extraction

The extraction methods of natural dye basically depend on medium in which the dye is extracted. There are mainly four methods used in extraction of natural dyes where they are aqueous method, alkaline method, acidic method and alcoholic method.

2.5.1.1 Aqueous Method

The known amount of dyestuff is boiled in 100 ml of soft water at 100°C. Then the dye solution is filtered and finally the optical density is recorded.

2.5.1.2 Alkaline Method

First, 1% alkaline solution is prepared with addition of 1g of NaOH in 100ml of water. Then the dye material is entered and boiled at 100°C. Finally the dye solution is filtered and the optical density is recorded.

2.5.1.3 Acidic Method

Acidic solution of 1 % is prepared by adding 1 ml of HCl in 10 ml of soft water. Then the material is entered and boiled at 100°C. Finally, the dye solution is filtered and the optical density is recorded.

2.5.1.4 Alcoholic Method

Alcohol of 50ml is added to 50 ml of water. Then the dye material is entered and boiled and finally the dye solution is filtered.

2.5.2 Extraction Technology

The extraction can be carried out in aqueous, acid or alkaline medium. At present, small scale producers/ manufacturers are using extraction technology method. Even the local dyers using more crude method for extraction using metallic flax and crude process in refined way using blender condenser, distillation plant and drier and crystallization unit with the capacity of 300 ton per year. The modern techniques of extraction are carried out with the use of extraction plant, reverse osmosis process and the latest is supercritical fluid extraction method. This method is very common in developed countries.

2.5.2.1 Solvent Extraction

This technique was developed just before the dawn of twentieth century. Now it has been commercialized in recent years. This technology has been improved to reduce waste generation and eco-effectiveness of extraction technology. Ultrasonic extraction followed by micro-wave extraction of solid finds extensive use mainly on organic solvents extraction

2.5.2.2 Supercritical Fluid Extraction

This is a further advancement making significant step over the use of conventional solvent extraction technology. It uses CO₂ as extraction media. This technique is used for the extraction of natural products in food, pharmaceuticals and chemical industries too.

It makes it possible to work at moderate temperature without affecting the organoleptic qualities and the active ingredients of the extracts obtained. Moreover, it makes it possible to obtain 100% natural extracts, completely free from extraction solvent residues. At the end of the extraction, an expansion phase which is achieved by reducing pressure causes the CO₂ to change from the supercritical state to the gaseous state which enables it to be removed completely from the CO₂ extract obtained.

2.5.2.3 Microwave Assisted Extraction Technology

This is a high-speed method used to selectively target compounds from various raw materials. The technology uses a microwave applicator as the energy source during solvent extraction. The advantage of this technology faster the processing, produce better yield, improve quality, lower energy consumption, reduce solvent level and low capital investments.